

PATHOGENICITY OF THE FUNGUS *ENTOMOPHTHORA CULICIS*
FOR ADULT MOSQUITOES: *ANOPHELES STEPHENSI*
AND *CULEX PIPIENS QUINQUEFASCIATUS*

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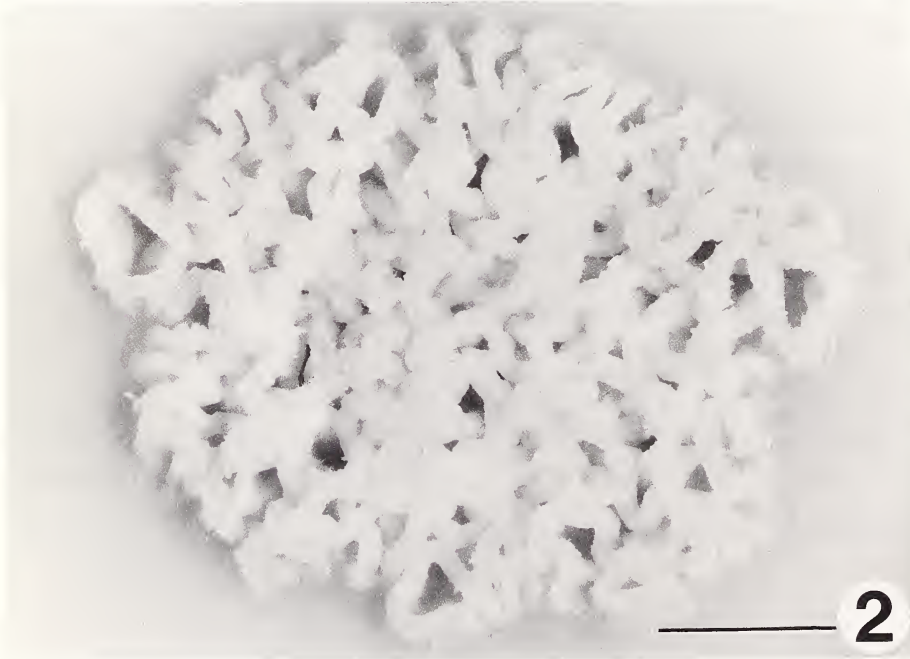
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Abstract.—The pathogenicity of *Entomophthora culicis* for the mosquitoes *Anopheles stephensi* and *Culex pipiens quinquefasciatus* was determined by exposing healthy adults to conidial showers from cadavers of naturally infected *Chironomus decorus* and from cultures grown *in vitro*. While 100 percent of the *A. stephensi* succumbed to infection, only 20 percent of the *C. pipiens quinquefasciatus* did so. Explanations for these differences are suggested. Some morphological characteristics of the *E. culicis* used in this study are presented.

Adult *Aedes aegypti* exposed to conidia of the fungus *Entomophthora culicis* originating from field-collected cadavers of the midge *Chironomus decorus* or from experimentally infected *A. aegypti* or from yolk cultures can develop a fatal mycosis (Kramer, 1982). The present study extends our understanding of this fungus and its disease-causing abilities in two more medically important mosquito species; namely, *Anopheles stephensi* and *Culex pipiens quinquefasciatus*. The former is an important vector of malarial parasites from the middle East to India while the latter is a principal developmental host of the filarial worm *Wuchereria bancrofti* in Southeast Asia (Harwood and James, 1979).

MATERIALS AND METHODS

Sources of conidia. *E. culicis* conidia from two sources were used in this study. These were field-collected cadavers of naturally infected adult *Chironomus decorus* found in Ithaca, New York and mycelial mats produced from conidia discharged from *C. decorus* cadavers onto the egg-yolk medium of Müller-Kögler (1959). Fresh whole cadavers were placed on 1.5 percent water agar in small plastic dishes to promote the development of the fungus. The discharge of conidia commenced within 24 hours. Small chunks of mycelial mats taken from yolk cultures, 7 to 10 days old, were also placed on water agar. The discharge of conidia from these chunks usually began



within 48 hours. Dishes containing cadavers or chunks of mats were held at $20 \pm 2^\circ\text{C}$ in closed chambers containing moist filter paper.

Testing procedures. Adult mosquitoes 24 to 48 hours old from disease-free insectary cultures were placed in cylindrical transparent plastic cages (height 35 mm, width 30 mm), having two large mesh-covered windows and mesh-covered tops and bottoms. A small plastic dish containing water agar with several cadavers or chunks of mycelial mats was inverted over each cage to allow the conidia being discharged to fall through the mesh-covered top into the cage of mosquitoes. These cages with dishes were housed in glass battery jars containing a layer of wet sand. The jars, tightly closed with polyethylene wrap, were held in cabinets at $20 \pm 2^\circ\text{C}$ with an 18:6 photoperiod. After 48 hours the mosquitoes were transferred to glass-covered carton cages provisioned with bottles of a 3 percent sucrose solution. These cages, held at $20 \pm 2^\circ\text{C}$ with an 18:6 photoperiod, were checked daily for dead mosquitoes. The dead ones were placed on water agar and examined for external growth of *E. culicis* at irregular intervals up to 48 hours. Only those cadavers that produced a bloom of *E. culicis* on their exteriors by the 48th hour, were scored as infected. Female and male mosquitoes were present in equal numbers in each test group. No attempt was made to determine whether one sex or the other was more susceptible because of the small numbers of individuals used in each test.

RESULTS AND DISCUSSION

Representative conidia of the *E. culicis* used in this study are depicted in Figure 1. The binucleate primary conidia are broadly ellipsoidal with a papillate apex and a flattened base. Their size depends upon the substrate from which they developed. Those discharged from cadavers were about 14 to 16 by 10 to 11 μm , while those produced *in vitro* were consistently larger, measuring about 17 to 19 by 12 to 15 μm . In both shape and size these primary conidia are virtually identical to the *E. culicis* conidia described by Gustafsson (1965) and others (see MacLeod et al., 1976). The primary conidium produces a binucleate secondary conidium which is ovoid with a rounded apex; such conidia range in size from 11 to 14 by 8 to 11 μm . Secondary conidia produced one to four long germ tubes which are sometimes branched. Tertiary conidia were not observed. Conidia discharged

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Figs. 1, 2. Fig. 1. Conidia of *Entomophthora culicis*. Note primary conidium surrounded by protoplasmic layer (at left), two secondary conidia with long germ tubes, and two primary conidia without protoplasmic layer. Bar equals 15 μm . Fig. 2. *In vitro* culture of *Entomophthora culicis* showing its typically convoluted growth pattern. Bar equals 0.75 mm.

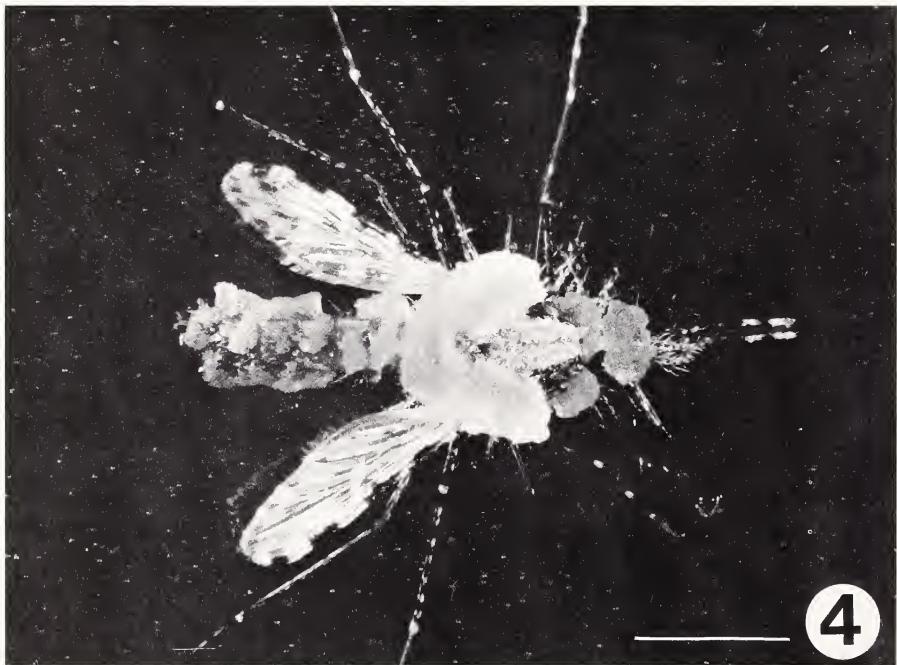
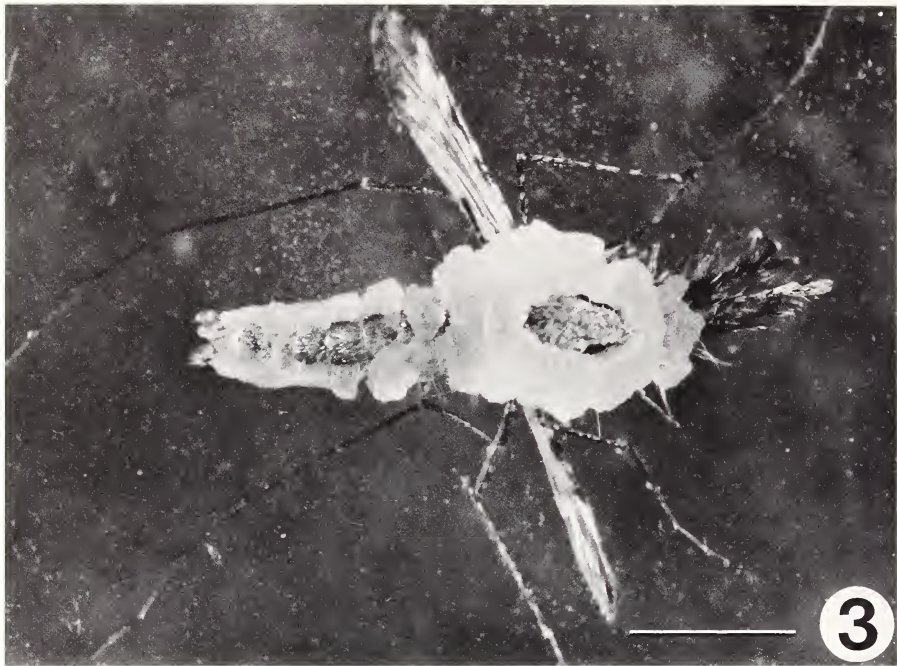


Table 1. Mortality at post-inoculation day 6 among adult mosquitoes experimentally infected with *Entomophthora culicis*.

Species of mosquito	Sources of conidia		
	<i>Chironomus decorus</i> cadavers	Egg-yolk cultures	Both sources
<i>Anopheles stephensi</i>	100% (18/18)	100% (33/33)	100% (51/51)
<i>Cx. p. quinquefasciatus</i>	11% (4/38)	8% (2/26)	9% (6/64)

onto yolk agar produce glassy, greyish-white cerebriiform colonies in 10 days to two weeks at $20 \pm 2^\circ\text{C}$ (Fig. 2). Chunks of these colonies placed on water agar generally developed a velvety hymenium after 24 to 48 hours.

Test mosquitoes and E. culicis. The post-mortem appearance of mosquitoes that succumbed to an *E. culicis* infection is shown in Figures 3 and 4. The fungal outgrowths so prominent on these cadavers occur only when the dead mosquito rests upon a moist surface. In nature midges with fatal *E. culicis* infections seek out shaded moist surfaces on which to die and thereby contribute to the survival and perpetuation of the agent responsible for their deaths (Kramer, 1981). It is conceivable that this pattern of behavior would also occur among mosquitoes with *E. culicis* infections in nature.

The results of the infectivity tests indicate that adults of both mosquito species are susceptible to infection by *E. culicis* (Table 1). The relative susceptibilities of these two hosts are, however, quite different. While 100 percent of the *A. stephensi* succumbed to infection by the end of post-inoculation day 6, only about 9 percent of the *C. p. quinquefasciatus* had done so in this time period. By post-inoculation day 10 mortality in the *Culex* group reached about 20 percent; many adults in this group lived for more than 10 days but never developed the mycosis. The observed differences in susceptibility may be attributed in part to the fact that *A. stephensi* is the smaller and more delicate of the two species at risk. *A. stephensi* may also be less efficient in dislodging conidia during the grooming process. Whether conidia originated from a cadaver or from an artificial substrate did not alter their relative ability to fatally infect host mosquitoes (Table 1). The time of death of infected *Anopheles* ranged from 3 to 6 days with 50 percent dying on day 3 (Table 2). For the *Culex* that became infected the

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Figs. 3, 4. Cadavers of *Anopheles stephensi* displaying post-mortem changes caused by *Entomophthora culicis*. 1. Male with typical wreath-like growth of fungus surrounding the thorax. Bar equals 2.5 mm. 2. Female with atypical crescent-like growth around posterior part of the thorax. Bar equals 3.0 mm.

Table 2. The temporal distribution of deaths among adult mosquitoes dying of the mycosis caused by *Entomophthora culicis*.

Species of mosquito	Number infected	Post-inoculation days of death*									
		1-2	3	4	5	6	7	8	9	10	11-30
<i>Anopheles stephensi</i>	(50)	0	50	8	18	24	0	0	0	0	0
<i>Cx. p. quinquefasciatus</i>	(24)	0	0	0	25	8	42	0	17	8	0

* Daily distribution in percentages.

time of death ranged from 5 to 10 days with 50 percent mortality occurring by day 7 (Table 2). If these patterns of mortality took place in nature, they would enhance the pathogen's chances for survival by keeping a supply of fresh conidia in the habitat of potential hosts over a period of several days.

In his review of methods for the mass production of *E. culicis* and other pathogens, Nolan (1981) suggests that this fungus may find place in the applied control of black flies. His conjecture can be expanded to include mosquitoes as well.

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